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Original article

Relationship between moderate food restriction during pregnancy and Fe, Zn and Cu contents in maternal tissues and foetuses

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Summary — This study was undertaken to monitor Fe, Zn and Cu contents in several maternal tissues and the products of conception of rats submitted to moderate food restriction during pregnancy. Two groups of female rats, nonpregnant (NP) and pregnant (P1), were fed ad libitum. A second group of pregnant rats (restricted diet, P2) was fed for 14 days with the same intake as NP, then the intake was increased by 5% from day 14 to day 21. A significant decrease in liver Fe content was noted in P1 and P2 pregnant rats on day 21, being more marked in livers of P2. Increases in Cu and Zn contents in liver were induced during pregnancy, but the values were significantly lower in P2 than in P1. On day 21, no significant differences due to food restriction were noted in serum concentrations of these trace elements, placental weight and placental Fe. However, dam body weight gain, placental Zn and Cu contents were reduced in P2. Foetuses of undernourished dams exhibited at term lower weight and smaller amounts of Fe, Zn and Cu than those in P1. These results confirm that moderate food restriction during pregnancy produces intrauterine growth retardation and that newborns have low trace element contents (particularly in Cu), which make them dependent on correct postnatal supply.

iron / zinc / copper / undernutrition / pregnancy / rat

Résumé — Relation entre une restriction alimentaire modérée pendant la gestation et les teneurs en fer, cuivre et zinc des tissus maternels et des fœtus. *Une étude a été réalisée pour analyser l'influence d'une restriction alimentaire modérée pendant la gestation sur l'évolution de la teneur en fer, zinc et cuivre dans certains tissus maternels et produits de la gestation. Deux groupes de rattes, gestantes (P1) et non gestantes (NP), ont été alimentés ad libitum. Pour un autre groupe de rattes gestantes (P2), l'alimentation a été restreinte : le régime était identique à celui du groupe NP pendant les 14 premiers jours, puis la ration a été accrue de 5 % du 14^{e} au 21^{e} jour. On a observé des diminutions significatives du contenu total en fer dans les foies des rattes gestantes P1 et P2, avec une diminution plus marquée dans les foies de P2. La gestation a entraîné une augmentation du contenu en Cu et Zn, hépatique, mais les valeurs sont significativement plus basses pour P2 que pour P1. Au 21^{e} jour, il n'existait pas de différences significatives dues à la restriction alimentaire, ni sur les concentrations sériques des éléments traces, ni sur le poids des placentas et leur contenu en Fe. Cependant, les conte-*

nus en Zn et Cu étaient plus bas dans les placentas de P2. À la fin de la gestation, les fœtus des rattes dénutries présentaient un poids et des quantités de Fe, Zn et Cu plus faibles que ceux de P1. Ces résultats confirment que la restriction alimentaire modérée pendant la gestation entraîne un retard dans le développement intra-utérin et que les nouveau-nés présentent de faibles stocks en éléments traces (plus particulièrement en Cu), ce qui les rend plus dépendants d'une alimentation postnatale correcte.

fer / cuivre / zinc / sous-nutrition / gestation / ratte

INTRODUCTION

A number of reports indicate that undernutrition during pregnancy can have negative effects in the mother as well as in the newborn (Munro et al, 1983; Pond and Mersmann, 1988; Rodríguez et al, 1991; Lumey, 1992; Rosso and Salas, 1994). In nutritional deficit conditions, various metabolic adjustments make possible the transport of nutrients to the foetus at the cost of the maternal tissues. However, there is a limit to this transfer and, at a certain point, the mother protects herself (Masters et al, 1983).

Although malnutrition in pregnancy is most prevalent in developing countries, in industrialised countries women may suffer from nutritional deficiencies as a result of unsuitable dietary restrictions aimed at controlling weight and preventing obstetric problems during delivery. Such deficiences may result in intrauterine growth retardation and low birth weight (Rasmussen and Fellows, 1985; Leury et al, 1990; Rodríguez et al, 1991).

Tests with laboratory animals have in general been carried out by applying very severe food restrictions throughout the entire pregnancy or at certain intervals during pregnancy (Pond and Mersmann, 1988; Sterin et al, 1989; Firmansyah et al, 1989); however, there is little information on moderate diet restrictions varying over the period of gestation which reproduce more faithfully the real conditions of the pregnant woman in our environment. An indiscriminate reduction of food reducing calorie and protein provision may also affect the intake of micronutrients unless the quality and variety of the components of the diet are taken into account. In order to avoid this, foods with a high density of specific nutrients such as trace elements should be chosen or suitable supplements taken. Special care should be taken with elements that do not meet requirements, such as Zn (Instituto Nacional de Estadística, 1995) or Fe (Carreti et al, 1992; Thomsen et al, 1993).

It is well known that maternal Fe deficiency anaemia increases preterm delivery and low birth weight (Scholl and Hediger, 1994), and severe Zn (Masters et al, 1983; Apgar and Everett, 1991) or Cu (Hall and Howell, 1969) deficiencies result in embryonic death, small foetuses and malformations.

Since all indiscriminate dietary restriction involves a lower supply of trace elements, our aim was to study the effects of moderate dietary restriction during pregnancy on maternal tissues and foetus trace element composition.

MATERIALS AND METHODS

Animals and diet

Eighty female virgin Wistar rats with an initial body weight of 160 ± 2 g (mean ± SEM), were kept in an environmentally-controlled chamber maintained at 20–22 °C, with a 12 h light/12 h

dark cycle and 55–70% humidity. A semi-synthetic diet was prepared according to the recommendations of the National Research Council (1978) (table I).

Experimental procedure

The rats were kept in stainless-steel cages with grid bottoms for a 5 day adaptation period. They were then mated with adult males from 1700 to 0900 hours (two females per male rat). The 24 hour period immediately following the appearance of copulation plugs was regarded as day 1 of pregnancy. Rats which did not become pregnant were used as control.

Two groups of rats, nonpregnant (NP, $n = 27$) and pregnant (P1, $n = 23$), were fed the diet and demineralised water (Milli-Q plus, Ultrapure Water System, Millipore Corporation, Bedford, MA, USA) ad libitum. Subsequently, another pregnant group (P2, $n = 22$) was restricted for the first 2 weeks to the intake level of the NP group. During the third week, they were provided with 5% more food than was consumed by the NP group. This percent increase corresponded to the intake increase in group P1 during the third week. This increase was done to avoid any serious effects occurring as a result of severe restriction regimens throughout pregnancy since the third week is the period of maximum food intake of rats under normal circumstances.

Table I. Composition of the diet.

Ingredients (g kg^{-1})	
Casein	158
DL-methionine	2
Cellulose	53
Wheat starch	374
Sucrose	346
Olive oil	50
Sunflower oil	5
Mineral mix [1]	38
Vitamin mix [2]	15
Chemical analysis (mg kg^{-1} dry matter)	
Iron	90
Zinc	41
Copper	12

[1] Contents (g kg^{-1} diet): $CaCO_3$, 10; $CaHPO_4$, 6.8; KH_2PO_4, 8.2; $KHCO_3$, 6.1; NaH_2PO_4, 2.3; $MgSO_4.7H_2O$, 2.3; $(MgCO_3)_4.(OH)_2Mg.5H_2O$, 0.89; NaCl, 0.90; $FeSO_4.7H_2O$, 0.20; $MnSO_4.H_2O$, 0.17; $ZnCO_3$, 0.03; $CuSO_4.5H_2O$, 0.03; NaF, 0.002; Na_2CrO_4, 0.001; Na_2SeO_3, 0.0002; KI, 0.0002. [2] Contents (mg kg–1 diet): choline chloride, 1 341; folic acid, 1; nicotinic acid, 20; calcium pantothenate, 8; riboflavin, 3; thiamin hydrochloride, 5.1; pyridoxine, 6; cyanocobalamin, 0.05; retinol (4 000 IU), 1.2; cholecalciferol (1 000 IU), 0.03; DL-α-tocopheryl acetate, 30; menadione, 0.07; wheat starch, 13 584 (as excipient).

Body weight was measured on days 7, 14, 18 and 21 of the assay. Several animals from each group were sacrificed on the same days. Laparotomies were performed under sodium pentobarbital (Abbott Laboratories, SA, Madrid, Spain) anaesthesia. Maternal blood was collected by cannulation of the carotid artery. Serum was obtained by centrifugation for 15 min at 900 x *g* (JP Selecta, SA, Barcelona, Spain) and stored in disposable plastic vials at –20 °C until analysis. Maternal liver and the right femur were removed and weighed. The products of conceptus were also removed, cleaned of adhering membranes and weighed. On day 21, individual foetuses were separated from their corresponding uterus-placenta complexes; five litters of P1 and eight litters of P2 were used to assess placental and foetus mineral content. Two foetuses were randomly chosen from each of the 13 litters studied and it was assumed that the mean concentration of trace elements obtained for the two analysed foetuses was representative of the entire litter. All tissues were stored frozen at –20 °C until trace element analysis.

Analytical methods

Diet samples (mean weight 1 g), the entire liver, the right femur, all the placentas and two individual foetuses per dam (randomly chosen) were dry-ashed in a muffle furnace and dissolved in 1.5 ml of a $HCl\text{-}HNO_3\text{-}H_2O$ solution (1 + 1 + 2, Suprapur, Merck, Darmstadt, Germany; ultrapure water). Afterwards, livers and placentas were diluted to 50 ml with ultrapure water while diet samples and foetuses were diluted to 25 ml and femurs to 250 ml. Additional dilutions were used as necessary to obtain concentrations in the linear reading range. Duplicated samples were analysed for Fe, Zn and Cu by flame atomic absorption spectrometry (Perkin-Elmer 1100B, Norwalk,

CT, USA). Aliquots of diet were used as internal control to assess precision. The interassay relative standard deviation was 4.0, 5.5 and 1.7% for Fe, Zn and Cu, respectively. Bovine liver (certified reference material CRM 185, Community Bureau of Reference, Brussels, Belgium) yielded values (mean ± SEM of five determinations) of: Fe, 210 ± 6 μg g^{-1}; Zn, 149 ± 5 μg g^{-1} and Cu, 186 ± 3 μg g^{-1} (certified values: 214 ± 5, 142 ± 3 and 189 ± 4 μg g^{-1} for Fe, Zn and Cu, respectively).

Statistical analysis

The data were analysed by two-way analysis of variance (ANOVA), for time (days 7, 14, 18, 21) and group (NP, P1, P2). However, since a significant interaction time x group was demonstrated, the two variables were analysed separately (one-way ANOVA for either time or group). Data were processed with the Biomedical Statistical Package (BMDP) (1992), BMDP 2D (descriptive analysis) and BMDP 7D (ANOVA and multiple range test), running VMS-DEC alpha 2100.

RESULTS

Daily food intake during the first 2 weeks was (mean ± SEM): 16.0 ± 0.8, 20.2 ± 0.9 and 15.6 ± 0.6 g day^{-1} in NP, P1 and P2, respectively, and during the last week 16.3 ± 0.7, 21.1 ± 0.9 and 17.0 ± 0.5 g day^{-1} in NP, P1 and P2, respectively. P1 values were significantly higher ($P < 0.05$) compared to the other groups and no significant differences appeared between NP and P2.

Body weight gain (fig 1) slightly increased in NP rats. Pregnant rats from the two groups showed a higher increase compared to NP, being the differences between P1 and P2 significant from day 7. Nevertheless, conceptus weight was only slightly lower in P2 compared to P1 but the differences did not reach statistical significance.

Maternal hepatic weight increased during pregnancy in P1 and P2 (table II). In P1, the differences with respect to NP were always significant ($P < 0.01$). However, in P2 differences with respect to NP only appeared on days 18 ($P < 0.01$) and 21 ($P < 0.01$). Livers of P2 were significantly smaller than those of P1 on days 7 ($P < 0.01$) and 21 ($P < 0.01$). Total liver Fe content did not vary in any of the groups until day 21 when the values of NP, P1 and P2 differed significantly ($P < 0.01$) (table II). In P2, liver Fe decreased by half between days 18 and 21.

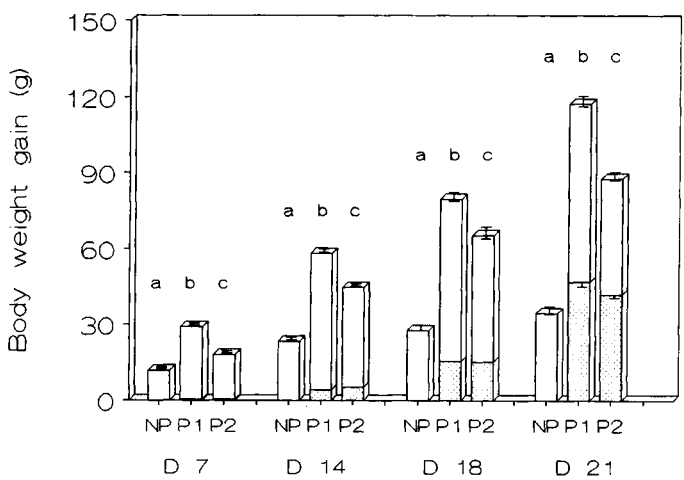


Fig 1. Body weight gain in nonpregnant (NP) rats, pregnant rats fed ad libitum (P1) and pregnant rats fed a restricted diet (P2). In pregnant rats the total bars are divided into conceptus weight (white area) and net dam body weight (dotted area). Values represented are mean ± SEM of four to eight animals per group. Within days (D), total bars not sharing a common letter (a,b,c) represent significant differences in body weight gain. Differences in conceptus weight between groups were not significant.

Table II. Iron, zinc and copper contents in livers of nonpregnant and pregnant rats fed a diet ad libitum and of pregnant rats submitted to food restriction.

	Weight (g)	*Fe*		*Zn*		*Cu*	
		μg	*μg g^{-1}*	*μg*	*μg g^{-1}*	*μg*	*μg g^{-1}*
Nonpregnant							
7 d (6)	7.8 ± 0.3^{ab}	1.46 ± 0.03	0.19 ± 0.01	200 ± 11^{ab}	25.7 ± 0.7	41 ± 1^{ab}	5.3 ± 0.2^{ab}
14 d (5)	7.6 ± 0.5^{a}	1.49 ± 0.06	0.20 ± 0.01	182 ± 8^{a}	24.0 ± 0.6	40 ± 2^{ab}	5.4 ± 0.3^{a}
18 d (4)	7.9 ± 0.2^{ab}	1.43 ± 0.07	0.18 ± 0.01	189 ± 7^{ab}	23.8 ± 0.5	36 ± 2^{b}	4.6 ± 0.1^{b}
21 d (10)	8.8 ± 0.2^{b}	1.50 ± 0.04	0.17 ± 0.00	219 ± 6^{b}	25.0 ± 0.5	43 ± 1^{a}	4.9 ± 0.1^{ab}
P day	0.0250	NS	NS	0.0127	NS	0.0187	0.0168
Pregnant ad libitum							
7 d (5)	9.4 ± 0.2^{a*}	1.39 ± 0.06	0.15 ± 0.01^{a*}	227 ± 3^{a}	24.1 ± 0.4^{a}	47 ± 1^{a}	5.0 ± 0.2^{a}
14 d (5)	10.3 ± 0.5^{a*}	1.46 ± 0.07	0.14 ± 0.00^{a*}	245 ± 14^{a*}	23.8 ± 0.4^{a}	56 ± 3^{b}	5.4 ± 0.1^{ab}
18 d (5)	10.8 ± 0.2^{a*}	1.44 ± 0.09	0.13 ± 0.01^{a*}	320 ± 9^{b*}	29.5 ± 0.9^{b*}	62 ± 0^{b*}	5.7 ± 0.1^{b*}
21 d (7)	13.7 ± 0.4^{b*}	1.23 ± 0.08*	0.09 ± 0.01^{b*}	342 ± 8^{b*}	25.0 ± 0.5^{a}	69 ± 2^{c*}	5.1 ± 0.1^{a}
P day	0.0000	NS	0.0000	0.0000	0.0020	0.0000	0.0087
Pregnant restricted							
7 d (4)	7.5 ± 0.2^{a†}	1.35 ± 0.07^{a}	0.18 ± 0.01^{a}	192 ± 6^{a†}	25.4 ± 0.5^{ab}	43 ± 3^{a}	5.8 ± 0.5
14 d (4)	8.8 ± 0.3^{a}	1.34 ± 0.06^{a}	0.15 ± 0.01^{ab*}	245 ± 5^{b*}	27.9 ± 0.6^{a*†}	50 ± 0^{ab*}	5.7 ± 0.1
18 d (4)	10.2 ± 0.4^{b*}	1.42 ± 0.08^{a}	0.14 ± 0.00^{b*}	255 ± 12^{b*†}	25.0 ± 0.7^{b†}	60 ± 0^{c*}	5.9 ± 0.2*
21 d (6)	10.1 ± 0.2^{b*†}	0.75 ± 0.05^{b*†}	0.07 ± 0.01^{c*}	238 ± 6^{b†}	23.6 ± 0.6^{b}	51 ± 2^{b*†}	5.1 ± 0.1
P day	0.0000	0.0000	0.0000	0.0002	0.0012	0.0000	NS

Values are mean ± SEM; the number of animals for each day is indicated in parentheses. Within each group, when a significant influence of day (d) was found, common superscript letters indicate the groups of identical values. * Significantly different from the corresponding nonpregnant value ($P < 0.05$); † significantly different from the corresponding pregnant ad libitum value ($P < 0.05$); NS: nonsignificant.

Liver Fe concentration decreased regularly and was lower in the two pregnant groups than in NP without significant differences between P1 and P2. The total content of Zn varied irregularly along the experimental period in NP without variation in concentration. Total Zn in P1 and P2 livers increased during pregnancy, but to a lesser extent in P2 (ANOVA for group, time and interaction group x time showed highly significant effects, $P < 0.0000$). Liver Zn concentration in P1 and P2 was higher than in NP on days 18 and 14, respectively. Absolute and relative values of liver Cu in NP exhibited a slight decline on day 18 followed by recovery. Total liver Cu tended to increase during pregnancy. Differences in P1 and P2 compared to NP were significant from day 14, but livers of P2 rats reached the end of pregnancy with less Cu than those of P1 rats ($P < 0.01$). The concentration of this metal in livers of P1 and P2 rats showed an elevation at the beginning of the third week of gestation followed by a decline. Even though this gestational time effect was significant in P1 but not in P2, liver Cu concentrations were higher in the two pregnant groups than in NP on day 18 ($P < 0.01$).

Femur Zn showed a tendency to increase at the end of the experimental time in NP

($P < 0.0001$), P1 ($P < 0.01$) and P2 ($P < 0.01$) and the highest values were obtained on day 21; however, the differences between groups were not significant (fig 2).

The serum concentrations of Fe and Zn did not show significant differences between groups until day 21. On this day, serum Fe and Zn were lower in P1 and P2 with respect to NP. Serum Cu tended to increase during pregnancy and the differences between each pregnant group and NP were significant on days 18 and 21. Nevertheless, serum Fe, Zn and Cu levels did not differ between P1 and P2 (fig 3).

The incorporation of trace elements in the conception products was very small until day 14 and was accelerated in the last days (fig 4). On day 21, the differences between groups reached significant levels ($P < 0.05$ for Fe and Zn and $P < 0.01$ for Cu). Placental weight was similar in P1 and P2 (table III). Similarly, no significant influence of food restriction on Fe composition was detected. Nevertheless, Zn and Cu total contents in placentas were reduced by food restriction, although their relative values were unaffected. Food restriction did not alter litter size (table IV) but decreased foetus weight and whole foetus trace elements. The differences between groups for relative trace element content were only significant for Cu.

DISCUSSION

Results concerning food intake and body weight gain have previously been described (Vaquero and Navarro, 1993). The estimated caloric intake for the entire period was 1 280 Kcal for NP, 1 282 Kcal for P2 and 1 640 Kcal for P1. Therefore, the caloric restriction in P2 was 22% with respect to P1. This moderate food restriction induced a decrease in body weight gain and foetus weight. However, conceptus weight taken as a whole remained similar in both pregnant rat groups. This, in agreement with Rasmussen and Fellows (1985), confirms that under inadequate dietary restrictions the maternal organism (litter free) decreases in weight in favour of the conceptus.

As described previously (Williams et al, 1977; Rosso and Cramoy, 1979), liver weight increased during normal pregnancy. Malnourished rats showed the same trend although their liver weight was under the

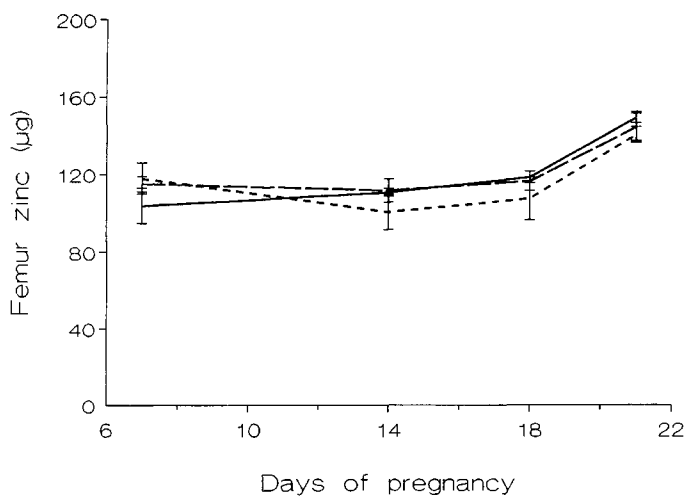


Fig 2. Femur Zn content in nonpregnant rats (solid line), pregnant rats fed ad libitum (dashed line) and pregnant rats fed a restricted diet (dotted line). Values represented are mean ± SEM of four to eight animals per group. The differences between groups were not significant. The influence of time was significant ($P < 0.01$).

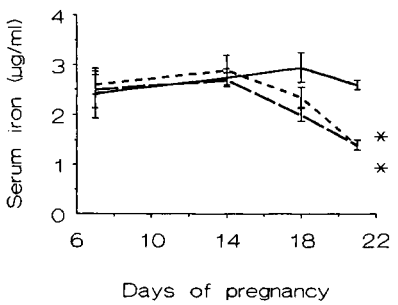
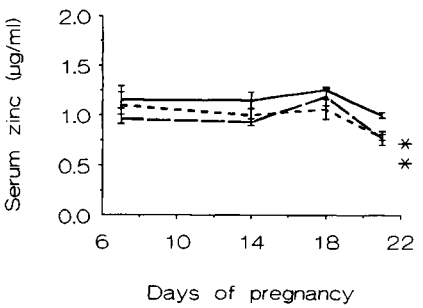
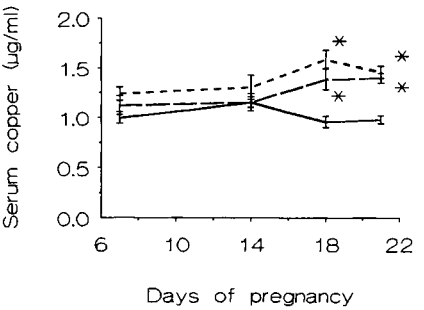


Fig 3. Iron, zinc and copper levels in serum of nonpregnant rats (solid line), pregnant rats fed ad libitum (dashed line) and pregnant rats submitted to food restriction (dotted line). Values represented are mean ± SEM of four to ten animals per group. * Significant differences from nonpregnant rats (NP) ($P < 0.05$).





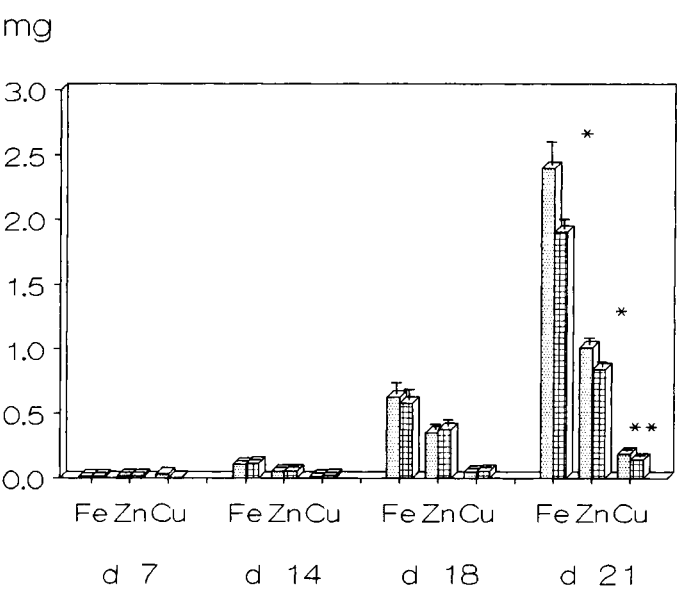


Fig 4. Influence of food restriction on conceptus mineral composition in rats. (Dotted bars) pregnant ad libitum; (checkered bars) pregnant restricted. Values represented are mean ± SEM for four to eight animals per group. *, ** Significant differences between groups at levels $P < 0.05$ and $P < 0.01$, respectively. d: day.

Table III. Influence of moderate food restriction on placental composition of rats on day 21 of pregnancy.

	Weight (g)	*Fe*		*Zn*		*Cu*	
		μg	*μg g^{-1}*	*μg*	*μg g^{-1}*	*μg*	*μg g^{-1}*
Pregnant ad libitum (5)	9.4 ± 0.5	939 ± 82	99 ± 3	144 ± 4	15 ± 1	44 ± 2	4.7 ± 0.4
Pregnant restricted (8)	8.6 ± 0.4	781 ± 47	91 ± 5	126 ± 7	15 ± 1	37 ± 2	4.3 ± 0.2
P group	NS	NS	NS	0.0382	NS	0.0407	NS

Values are mean ± SEM; the number of animals per group is indicated in parentheses. NS: nonsignificant.

Table IV. Influence of moderate food restriction on foetus composition on day 21 of pregnancy in rats.

	Litter size	*Foetus weight (g)*	*Fe*		*Zn*		*Cu*	
			μg	*μg g^{-1}*	*μg*	*μg g^{-1}*	*μg*	*μg g^{-1}*
Pregnant ad libitum (10)	9.8 ± 0.3	3.8 ± 0.1	148.7 ± 7.8	39.0 ± 1.9	89.1 ± 3.5	23.4 ± 0.9	15.0 ± 0.9	3.9 ± 0.2
Pregnant restricted (16)	10.0 ± 0.3	3.2 ± 0.1	112.8 ± 6.2	35.2 ± 1.7	72.3 ± 2.6	22.4 ± 0.4	10.7 ± 0.6	3.3 ± 0.1
P group	NS	0.0026	0.0014	NS	0.0008	NS	0.0004	0.0200

Values are mean ± SEM; two foetuses per litter were analysed; the total number of foetuses analysed per group is indicated in parentheses; NS: nonsignificant.

corresponding value of pregnant rats fed freely. This smaller liver size was the primary cause of the decreased content of trace elements in the organ, as described later.

The total liver Fe decrease at the end of pregnancy, which was more marked in the group subject to restriction, should be related to conceptus iron accretion that was very high during maximum foetal growth. Likewise, liver Fe relative values showed similar depletions of this metal in both gestation groups. However, total liver Fe was much lower in the underfed group due to differences in liver mass increases. Our results in rats are related to observations in humans concerning the drop in blood ferritin during late pregnancy, which in the opinion of Carretti et al (1992) is physiological. However, the higher reduction of stored liver Fe in P2 compared to P1 was related to the dietary restriction in this study.

The slight variations in liver Zn and Cu contents in NP rats seem to be independent of food intake, which was rather constant (Vaquero and Navarro, 1993), or body weight gain. They can be partly associated with differences in liver weight. However, the different number of livers analysed per day or other factors may have conditioned these results and they are interpreted with caution.

The elevation in total Zn content in maternal liver throughout pregnancy (P1) cannot be totally attributed to the enlarged organ, since on day 18 liver Zn concentration was higher in the ad libitum pregnant group than in the nonpregnant group. Restricting the feeding of the pregnant rats, P2 caused the rise in Zn liver content to be earlier and less marked. During rat gestation, Zn is concentrated in the liver and released on the last days (Herzfeld et al, 1985). However, when Zn levels do not fulfil foetal requirements, Zn release is anticipated. Our results are consistent with those by Herzfeld et al (1985) obtained with pregnant rats fed with a marginal Zn deficient diet and suggest that the assayed dietary regimen brought about a marginal Zn deficiency status.

However, femur Zn was not affected by pregnancy or food restriction. It has been previously described that in pregnant rats femur Ca was higher than in nonpregnant rats on day 7 and that apparent bone density increased by mid-pregnancy and decreased from then on (Vaquero and Navarro, 1993). It is known that bone is an effective calcium store, but it can only contribute endogenous Zn by reducing Zn uptake (Golden, 1989). Zinc-deficient diets have been reported to reduce osseous Zn (Herzfeld et al, 1985; Masters et al, 1986). In this study, the dietary restriction during pregnancy might have produced slight changes in femur Zn that resulted undetectable at the level of one femur (fig 2).

Total liver Cu increased throughout pregnancy, a change that was associated with the increase in liver mass and the tendency of concentrating Cu by day 18 in the ad libitum pregnant group. Under the restricted regimen, a reduction in liver Cu at the end of pregnancy was observed instead of further elevation in relation with differences in liver weight gain. Even so, the necessary foetal supply was not achieved. It is possible that the accumulation of copper in liver until day 18 of pregnancy was not clearly seen in P2 rats due to higher deviation of values from day 7. However, in group P2 liver copper concentration at each experimental time did not significantly vary from the corresponding of P1. Therefore, even though the variations in total liver copper in P2 appear associated to the changes in liver mass, a tendency of decreasing copper concentration at the end of gestation should not be ruled out.

Liver Zn and Cu concentrations exhibited similar evolution along pregnancy. Both metals interact with a variety of intracellular ligands including metallothionein (Cousins and Hempe, 1990). Stimulus such as stress and hormones such as glucocorticoids, catecholamin, glucagon, progesterone, etc, which stimulate hepatic metallothionein synthesis (Golden, 1989; Cousins and Hempe, 1990), may be responsible for the accumulation of Zn and Cu in liver. The hormonal fall that takes place near term, together with the high foetal demand, may favour the release of Zn and Cu from liver to foetuses, especially in the restricted group.

Maternal serum trace element levels were not affected by food restriction. The lower Fe and Zn concentrations and higher Cu concentrations compared to nonpregnant values have been widely described in the literature (Thauvin et al, 1992; Arnaud et al, 1993; Hollan and Johansen, 1993; Wasowicz et al, 1993). These results confirm the protective role of body stores under a moderate food restriction regimen.

Trace elements were slowly incorporated into the conception products up to day 18 and no effects due to the dietary regimen

were detected during this period. However, when the incorporation was enhanced, between day 18 and 21 (Greizerstein, 1982), trace element deficiency was shown in the undernourished group, resulting in conceptus with reduced Fe, Zn and especially Cu stores at term.

Even though the differences in placenta weight and placental Fe did not reach statistical significance, slightly smaller placentas in P2 contained less Zn and Cu than in P1, but the relative contents of both trace elements were unchanged. It is known that placental growth affects foetal development because the uptake of nutrients is limited (Malhotra et al, 1990). However, moderate food restriction in pregnant rats did not affect litter size nor produced preterm deliveries (on day 21 all foetuses were separated by laparotomy), features that have been associated with severe deficiencies of Fe (Scholl et al, 1992), Zn (Gibson, 1994) and Cu (Hall and Howell, 1969; Keen et al, 1982).

However, the general nutritional deficit induced by the dietary treatment led to reduced maternal weight and foetal growth retardation, which is consistent with many reports. Smaller amounts of trace elements were also found in these foetuses. However, only the concentration of Cu in their bodies decreased significantly. This trace element must be specially sensitive to restricted feeding, since the concentration of major minerals in the foetuses of undernourished rats was not affected (Vaquero, 1987; Vaquero and Navarro, 1993).

It seems therefore that several maternal tissues contributed to the supply of trace elements to undernourished foetuses. These foetuses were small but without Zn and Fe deficits. The fact that the foetuses of malnourished dams were selectively depleted in Cu agrees with Masters et al (1983), who pointed out that the accumulation of Cu is not dependent on maternal tissue catabolism but may be accounted for by dietary intake.

Foetal Zn concentration was similar in groups P1 and P2, which can be explained by increased Zn absorption in P2 dams (Vaquero and Navarro, 1991) and the extra Zn supply from maternal muscle catabolism due to underfeeding (Masters et al, 1986), in addition to the amounts removed from liver, bone or other organs.

Concerning Fe, it seems that the metabolic adjustments produced, including depletion of Fe stores in the mother, were just enough to achieve the deposition of Fe in these smaller foetuses, since foetal Fe concentration did not clearly decrease. Therefore, together with other authors (Southon et al, 1989; Barrada et al, 1991), we emphasise that a good maternal Fe status is necessary to accomplish normal Fe stores in the newborn.

In conclusion, undernutrition induced by the assayed food restriction was responsible for decreased foetal weight and thereby foetus trace element contents, but on the whole foetus trace element concentrations were not significantly different than those of normal foetus, except for Cu that exhibited higher sensibility and a more selective depletion. Consequently, the newborns might be provided with lower total body stores and be dependent on adequate postnatal nutrition.

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